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AUTORADIOGRAPHIC STUDY ON TISSUE LOCALIZATION OF [^{14}C]CUCURBITINE IN MICE*

LIANG You-yi (梁猷毅)**, Carolyn MARLOWE, William J WADDELL

(Department of Pharmacology and Toxicology, Health Sciences Center, University of Louisville, Louisville, KY 40292, USA)

ABSTRACT Cucurbitine (3-amino-3-carboxylpyrrolidine), is an anthelmintic amino acid isolated from the seeds of *Cucurbita moschata* Duch. Male, Swiss Webster mice were each administered iv 129 mg/kg (about 7 μCi) of [^{14}C]cucurbitine. At 0.33, 1, 3, 9, 24 h and 3 and 9 d after treatment the mice were briefly anesthetized with ether and frozen by immersion in dry ice/hexane. The mice were processed for whole-body autoradiography by the Ullberg technique; this process does not allow thawing or contact with solvents.

At 20 min after iv, the radioactivity was highly localized in the liver, kidney, dorsal root ganglion, tracheal cartilage and pancreas of the mice. The intense localization persisted through 24 h in these tissues except for the pancreas which showed a decrease in concentration with time. There was also a moderate level of radioactivity in the nasal epithelium, GI mucosa, esophagus, salivary glands, thymus, bone marrow and Harder's gland. By 24 h there was a marked retention of radioactivity in liver, cartilage and dorsal root ganglion. On 3 d the highest concentration was in cartilage.

Since proline and hydroxylproline are important components of cartilage and since cucurbitine is structurally similar to these amino acids, the mechanism of its retention in cartilage may involve incorporation of [^{14}C]cucurbitine into the protein matrix. The localization in dorsal root ganglia may be linked to the altered motor activity seen after high doses.

KEY WORDS [^{14}C]cucurbitine; 3-amino-3-carboxylpyrrolidine; whole-body autoradiography; tissue localization; cartilage; dorsal root ganglion; mice

The pumpkin seed has traditionally been used in Chinese medicine for the treatment of helminthiasis (e.g. tape worm), but the active principle and mechanism of anthelmintic activity were not known. It was also found that pumpkin seeds inhibited the growth of immature *Schistosoma japonicum*[1].

A new amino acid, cucurbitine (3-amino-3-carboxylpyrrolidine), has been isolated from the seeds of *Cucurbita moschata* Duch[2]. It was synthesized[3] and also can be labeled with ^{14}C. Cucurbitine is a water soluble amino acid which has protective activity against schistosomal infection[4]. A preliminary distribution and elimination study was performed by analysis of several tissues; the radioactivity was found to be concentrated in the liver and kidney. This present investigation was performed in an effort to elucidate further the localization of [^{14}C]cucurbitine by whole-body autoradiography (WBAR).

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** Visiting researcher from Shanghai Institute of Materia Medica, Chinese Academy of Sciences, People's Republic of China.

MATERIALS AND METHODS

Adult ♀ Swiss Webster mice weighing 33—41.5 g were given standard food and water *ad libitum*. [^{14}C]Cucurbitine was synthesized at the Shanghai Institute of Materia Medica (specific activity of 1.5 μCi/mg). Mice were injected iv into the tail vein with a solution containing [^{14}C] cucurbitine 36.3 mg/ml normal saline. Mice were each given an average dose of 7 μCi of [^{14}C]cucurbitine, which corresponded to a dose of 129 mg/kg. The mice were killed at 0.33, 1, 3, 9, 24 h and 3 and 9 d after iv by first lightly anesthetizing the mice with ether and immersing it in a mixture of hexane and dry ice (−75℃).

It was necessary to trim and remove most of the hair from the mice to embed it. A 1.5% solution of carboxymethyl cellulose was used as the mounting medium for embedding. The completely frozen mice were then sagittally sectioned in a Slee cryostat at −20℃. In order to take the 20 μm- and 40 μm-thick sections, pieces of #800 Scotch tape were applied to the surface of the block before a section was cut; thus the cut section adhered to the tape. Sectioning was improved by gently pressing a sawed-off brush against the back of the tape just at the junction of the knife edge. The sections were allowed to dry for 1 wk in a freezer. The freeze-dried sections were placed against Kodak AA x-ray film and allowed to remain in light-tight containers approximately 4 wks to 1 yr at −14℃. These procedures, as originally described by Ullberg[5], did not allow thawing or contact with any solvents. Thus, there was no translocation or loss of radioactivity; the method has been described in detail previously[6].

To identify the areas of high concentration observed in cartilage or dorsal root ganglia, some sections were stained with a methyl green-pyronin solution 3 min and washed 2 min in alcohol[7]. Histological verification of the heavy sites of radioactive localization seen in the autoradiographs was then possible.

RESULTS

Prints of autoradiographs from the mouse frozen 20 min after iv [^{14}C]cucurbitine are shown in Fig 1 A, B. By this time the radioactivity had largely disappeared from the blood. The highest concentrations were seen in liver, kidney, pancreas, tracheal cartilage and dorsal root ganglion. This intense concentration of radioactivity persisted through 24 h in these tissues except for the pancreas which showed a decrease in radioactivity with time (Fig 1 C-E). A moderate level of radioactivity was noticed in the nasal epithelium, gastrio-intestinal mucosa, thymus, esophagus, salivary glands, bone marrow and Harder's gland. There was also a little radioactivity in the spleen, adrenal, lens of the eye, and melanin as well as in the lung and myocardium. Traces of radioactivity were detected in liver, brain, spinal cord and muscle at 3 d and 9 d intervals.

The localization in the intervertebral disk was more persistent than other sites of localization (Fig 1 D-F). In contrast, relatively low levels of radioactivity were seen in the vertebral bone, which is richly supplied with blood; this vertebral bone showed a decrease in concentration with time (Fig 1 B, E). Based on the areas of relatively high localization of radioactivity (trachea, epiglottis, rib and external ear cartilages), cucurbitine is preferentially taken up by the cartilaginous tissue.

Although there was no radioactivity in the spinal cord at 20 min through 9 h, the dorsal root ganglia were seen to be persistent sites of localization. There were aggregates of sensory neurons located within the ganglia that stained with methyl green-pyronin which corresponded precisely with Fig 1 A, B, D,

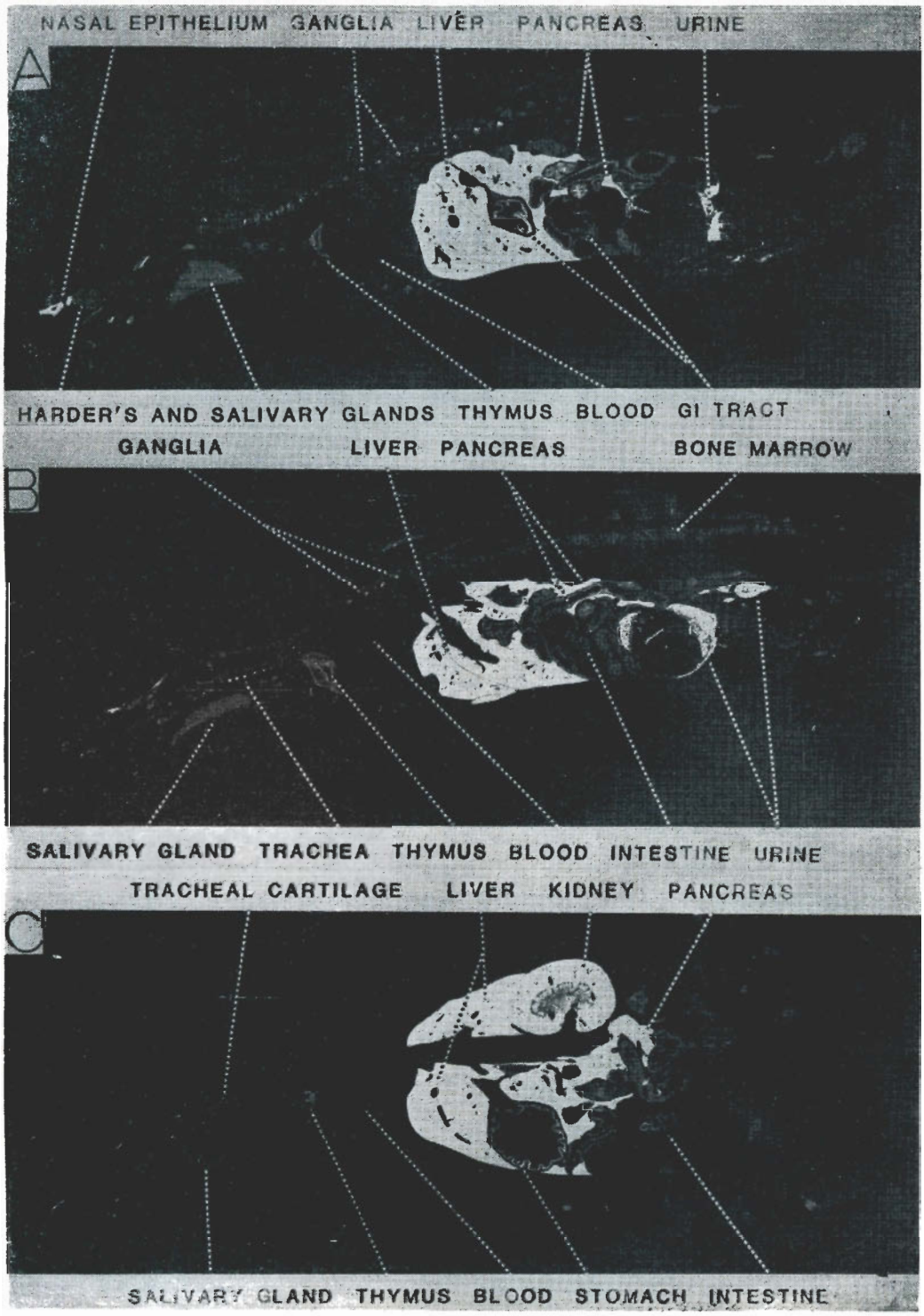
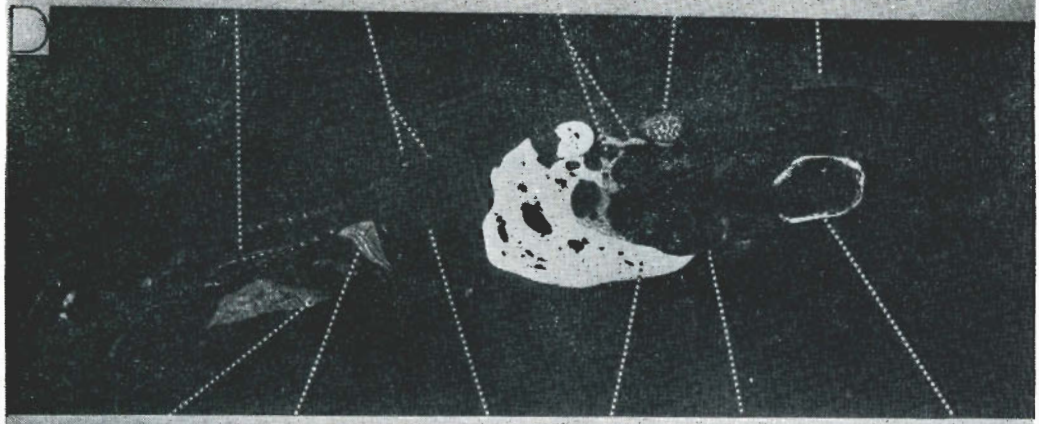


Fig 1. Autoradiographs of 20-μm-thick sections from ♂ mice which received iv [^{14}C]cucurbitine 129 mg/kg. White areas correspond to radioactivity. A,B) frozen 20 min after iv; C) 1 h after iv; D) 3 h after iv; E) 9 h after iv; F) 24 h after iv.

CARTILAGE GANGLIA PANCREAS KIDNEY BONE MARROW

D



SALIVARY GLAND THYMUS BLOOD LIVER INTESTINAL WALL URINE

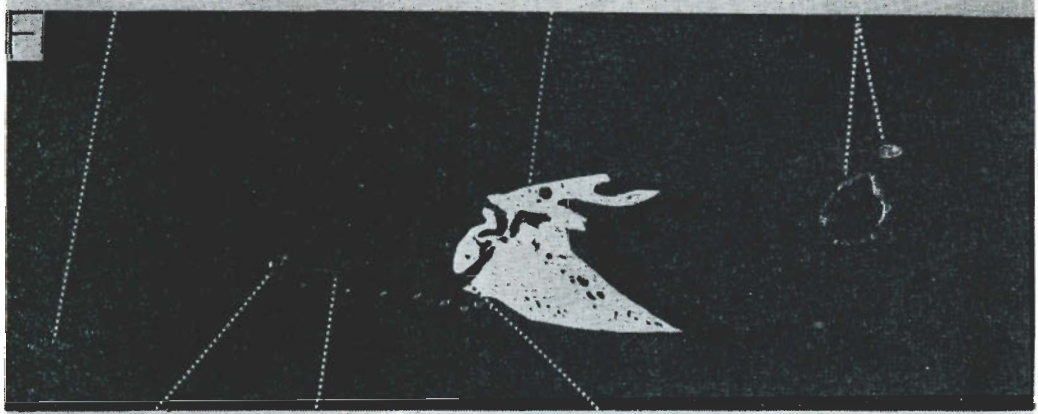
LENS INTERVERTEBRAL DISK BLOOD LIVER PANCREAS

E

CARTILAGE THYMUS MYOCARDIUM BILE URINE

LENS LIVER URINE

F



CARTILAGE THYMUS GALL BLADDER

DISCUSSION

The general distribution of [^{14}C] cucurbitine in mice was previously studied by homogenate tissue assays. The highest concentrations were found in liver, kidney and small intestine. Using the technique of WBAR we have found more specific sites of localization of this compound. Rapid and persistent accumulation of radioactivity was found in the spinal ganglion and certain cartilaginous tissues.

Cucurbitine is one of the naturally occurring amino acids. Its structure is similar to proline which is an important component of cartilage[8]. Hexamethonium and other quaternary ammonium compounds were distributed in cartilage[9]. It is hypothesized that these compounds are bound to the polyanionic mucopolysaccharide components of cartilaginous tissue. In view of the difference in chemical structure, the mechanism of retention of radioactivity from cucurbitine in cartilage may be different. It is likely that cucurbitine is incorporated into the protein of cartilage.

The high concentration of radioactivity in the kidney and urinary bladder at all time intervals probably reflects this as the main excretory pathway. Secretion by the proximal tubules probably helps to maintain high urinary concentrations. The liver is another organ which shows a high level of retention. Since the liver is so metabolically active the radioactivity observed in this organ may be due either to metabolism or to the incorporation of this amino acid into cellular proteins. In contrast, relatively little or no radioactivity was observed in the gall bladder, bile and intestinal contents. Thus, bile is not a prominent route of elimination of this compound.

As more radiolabeled compounds are investigated by WBAR more correlations are possible between the accumulation of radioactivity at specific sites and pharmacological effects[10]. Shiao *et al*[4] indicated that mice receiving higher dosages of cucurbitine showed a decrease in motor activity, lost their balance and lifted their tails. The mice often developed transient convulsions, arching of the back and stretching of forelimbs followed by generalized tremor 4 h after injection; they exhibited hyperirritability even after 24 h. The rapid, high concentration of radioactivity from this compound in the dorsal root ganglion may be an important link to the altered motor activity.

The clinical significance of the high concentrations of radioactivity in cartilage and spinal ganglion is not known. Cucurbitine itself has not as yet shown any therapeutic effects except anthelmintic activity, but possible effects on cartilage or spinal ganglia should be considered.

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